

Reproduction of *Xenodon dorbignyi* on the north coast of Rio Grande do Sul, Brazil

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*In memoriam

Information on sexual maturity, reproductive cycle, fecundity and sexual dimorphism of *Xenodon dorbignyi* was obtained from 537 individuals captured in a 333 ha sand dune area on the north coast of Rio Grande do Sul, Brazil, and from dissection and analysis of gonads of 98 specimens from the same region deposited in scientific collections. Females and males reach sexual maturity at about 260 mm and 220 mm snout–vent length (SVL), respectively. Males reach sexual maturity in their first year, whereas some females mature in their second year. Both sexes reach similar SVL; males have a relatively longer tail, and mature females have a heavier body. Reproduction is seasonal, with vitellogenesis occurring from August to January, mating from August to December, oviposition from November to February, and hatching from January to April. Clutch size varied from three to 10 eggs and was correlated with maternal SVL. The ratio between clutch mass and female total mass varied between 0.19 and 0.42 (>0.30 in 80% of observations).

Key words: Dipsadidae, natural history, reproductive cycle, Serpentes, sexual dimorphism, southern Brazil

INTRODUCTION

The life history of an organism is characterized by reproductive mode, reproductive frequency, clutch and litter size, size of newborns and age and size at sexual maturity (Pough et al., 2004). As a consequence of different reproductive costs, the age and size at which individuals mature can vary both inter- and intraspecifically (Parker & Plummer, 1987), leading to sexual size dimorphism at the adult stage (Shine, 1993). Fecundity is an important determinant for female reproductive success (Bonnet et al., 2001; Lourdais et al., 2003), in addition to timing of oviposition and quality of offspring (e.g. Olsson & Shine, 1997; Madsen & Shine, 1998). High reproductive output can be limited by environmental, genetic, behavioral, physiological and morphological factors (Williams, 1966; Shine, 1992).

Xenodon dorbignyi (Duméril, Bibron & Duméril, 1854) is an oviparous dipsadid snake (tribe Xenodontini) distributed in Paraguay, Argentina, Uruguay and the southernmost parts of Brazil, where it occupies open environments (Gudynas, 1979; Lema, 1994; Tozetti et al., 2010). Information on its reproductive biology mostly comprises records of clutches, hatchlings born in captivity and occasional observations of mating, mainly from populations in Uruguay and Argentina (e.g. Orejas-Miranda, 1966; Gallardo, 1977; Gudynas, 1979; Leitão-de-Araújo, 1978); Pontes & Di-Bernardo (1988) presented the only reproductive data available for populations from Rio Grande do Sul.

This study presents information about sexual maturity, sexual dimorphism, reproductive cycle and fecundity of *Xenodon dorbignyi* on the north coast of Rio Grande

do Sul, gathered from a large number of observations in nature and from dissections of specimens deposited in collections.

MATERIALS AND METHODS

The northern coast of Rio Grande do Sul is mainly characterized by a plain of mobile dunes, interposed by small humid depressions and patches with scarce vegetation, mostly Graminae (Waechter, 1985). The climate is temperate, with mean, minimum and maximum monthly temperatures ranging between 15.4, 12.2 and 18.3 °C in July, and 24.8, 21.3 and 27.6 °C in February, respectively. Rainfall is almost uniform across the year, with a small peak during winter and a mean of 1323 mm (Hasenack & Ferraro, 1989).

Field observations were carried out from July 1998 to June 2004 in a 333 ha area of sand dunes located in Magistério (30°21'S, 50°17'W), municipality of Balneário Pinhal.

Complementary information was gathered from 98 specimens (30 males and 68 females) collected locally and deposited in the herpetological collection of the Museu de Ciências e Tecnologia of Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Fundação Zoobotânica do Rio Grande do Sul (MCN), and Departamento de Zoologia of Universidade Federal do Rio Grande do Sul (DZUFRGS) (Appendix 1). Specimens from collections were dissected, recording snout–vent length (SVL), tail length (TL), sex, number of eggs or vitellogenic follicles, size of the larger follicle or egg (in mm), and condition of deferent ducts. Individuals were considered mature when they had vitellogenic follicles

larger than 8 mm or eggs in oviducts (females), or when they had convoluted and opaque deferent ducts (males, cf. Shine, 1982). For ambiguous specimens, transverse slices of the median region of the testis were prepared on slides, stained with haematoxylin-eosin and analysed for the presence or absence of spermatozoa. Maturity of live individuals was inferred based on SVL.

Sexual dimorphism was evaluated based on 537 live specimens (260 males and 277 females), and on 100 specimens (40 males and 60 females) born in captivity from gravid females captured in the study area. All individuals were measured (SVL and TL, using a ruler), weighed (in grams, using Pesola spring scales), and sexed by forced eversion of the hemipenes. The animals were individually marked by branding ventral scales with a burning wire (cf. Di-Bernardo et al., 2007), and released at their site of capture. After the branding process, marks were treated with antiseptic spray and an anaesthetic (benzalkonium chloride plus lidocaine) to avoid infection by pathogens. Only data from the first capture were used. In order to avoid the inclusion of possibly gravid females in the body mass analysis, only data from mature individuals collected from March to July (out of the reproductive season) were included. Since the SVL of both mature males and females violated the assumptions of equality of variance, an adjusted Student's *t*-test was used (*t'*) to compare their means (cf. Zar, 1999). Differences in TL and body mass were investigated by comparing their regressions in relation to SVL. The homogeneity of slopes was compared through analyses of variance (ANOVA), and the intercepts were compared with each other using covariance analysis (ANCOVA) after *ln* transformation of data.

The vitellogenic period was inferred from the seasonal distribution of the larger follicles of dissected females. The mating season was determined from observations of mating pairs. Gravid females were kept until oviposition (1–15 days at 24 °C), before release at their capture sites. These females were maintained individually in boxes of polyethylene sterilized with iodine solution. Eggs were counted and weighed within 48 hours of oviposition, and incubated in humid, sterilized vermiculite at about 28 °C. Hatchlings were counted, measured, weighed, marked and released at their mother's capture sites after they hatched. Relative clutch mass (RCM) was calculated as the ratio between clutch mass and female total mass (including the clutch mass) (cf. Seigel & Fitch, 1984). The relationships between the size of eggs, hatchlings and females were investigated using linear regression.

Reproductive frequency was estimated from the proportion of gravid females found during the reproductive season (cf. Plummer, 1984). Only captures from November were used because in October many females did not have well developed follicles that could be detected by palpation, and in December several females had already laid eggs.

Distributions were tested for normality and homogeneity of variances using Kolmogorov–Smirnov and Levene tests, respectively. All analyses used $\alpha=0.05$. Mean values are presented with ± 1 standard deviation. Statistical analyses were performed with SPSS 10.0 for Windows (SPSS Inc., 1999).

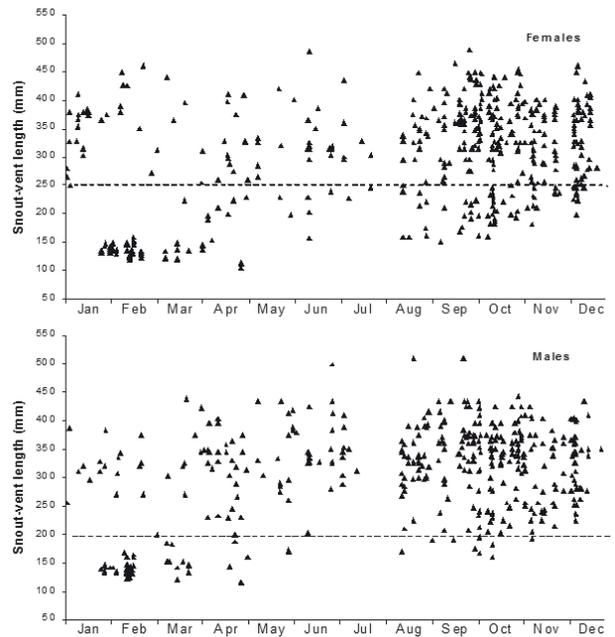


Fig. 1. Seasonal distribution of snout-vent length of females (above) and males (below) of *Xenodon dorbignyi*. The stippled line represents the snout-vent length of the smallest mature individual.

RESULTS

Sexual maturity

SVL of females ($n=67$ preserved specimens) varied between 178 mm and 469 mm; the smallest mature female was 257 mm. SVL of males ($n=31$) varied between 160 mm and 451 mm; the smallest mature male was 192 mm. One of two immature males had an SVL of 207 mm, which was larger than the SVL of two mature males. All males above 207 mm SVL were sexually mature.

The seasonal distribution of body size classes indicated that males attain sexual maturity in the first reproductive season after their birth (Fig. 1). The capture of small individuals in December suggests that some females do not attain sexual maturity in the first reproductive season (Fig. 1).

Sexual dimorphism

SVL of females ($n=337$) varied between 104 mm and 490 mm, and SVL of males ($n=300$) varied between 114 mm and 500 mm (Fig. 2). There was no significant difference between the SVL of newborn females and males ($n=60$ ♀ and 40 ♂; $\bar{x}=134.9\pm 10.9$ mm and 136.8 ± 10.9 mm, respectively; $t=0.85$, $df=98$, $P=0.397$). The mean SVL of mature females was significantly higher than that of mature males ($n=188$ ♀ and 239 ♂; $\bar{x}=351.1\pm 45.0$ mm and 321.3 ± 57.20 mm, respectively; $t=6.04$, $df=425$, $P<0.001$), although individuals of both sexes attain similar maximum SVL (Fig. 2).

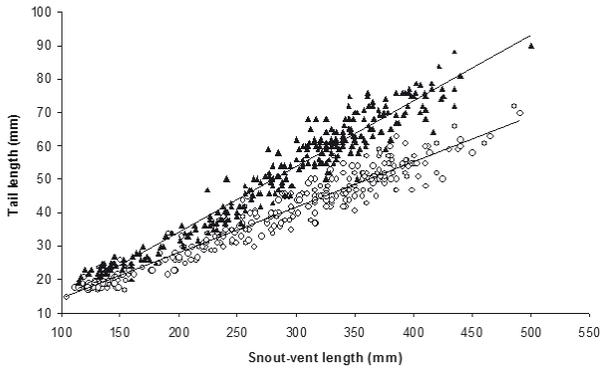


Fig. 2. Relationship between tail length and snout-vent length of female (circles) and male (triangles) *Xenodon dorbignyi*.

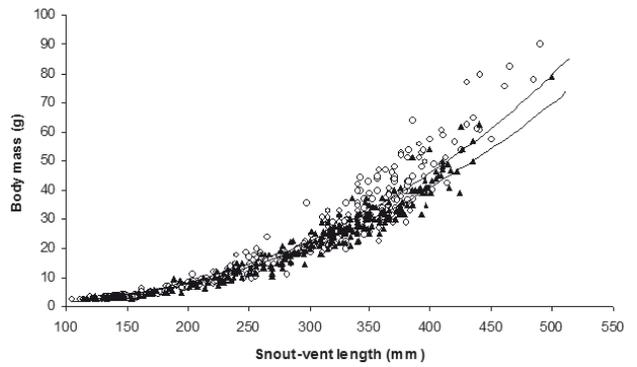


Fig. 3. Relationship between body mass and snout-vent length of female (circles) and male (triangles) *Xenodon dorbignyi*.

Males had significantly longer tails than females in all body size classes (Fig. 2). Taking only newborns into consideration ($n=60$ ♀ and 40 ♂), the regressions of \ln TL on \ln SVL did not differ in slope between the sexes ($F_{1,96}=1.37$, $P=0.245$), but were significantly different in their intercepts ($F_{1,97}=179.86$, $P<0.001$); males had proportionally longer tails than females. Considering mature individuals ($n=188$ ♀ and 238 ♂), the regressions had different slopes ($F_{1,422}=17.49$, $P<0.001$). After reaching maturity, the growth of the tail is proportionally higher in males than in females. Hatchling mass varied between 2.50 g and 4.50 g among males ($\bar{x}=3.44\pm 0.47$ g, $n=40$) and between 2.50 g and 4.65 g among females ($\bar{x}=3.37\pm 0.48$ g, $n=60$); there were no intersexual differences in slopes ($F_{1,96}=0.96$, $P=0.330$) and intercepts ($F_{1,97}=0.03$, $P=0.858$) when comparing regressions of \ln body mass against \ln SVL (Fig. 3). For mature individuals collected from March to July ($n=28$ ♀ and 56 ♂), such regressions did not differ in their slopes ($F_{1,80}=0.49$, $P=0.488$), but were significantly different with respect to their intercepts ($F_{1,81}=5.49$, $P=0.022$); females were relatively heavier than males at comparable SVL (Fig. 3).

Reproductive cycle

Vitellogenic follicles were recorded from August to January (Fig. 4, Table 1). Matings were recorded in August ($n=1$), September ($n=1$), October ($n=4$), November ($n=1$) and December ($n=1$, Table 1). Gravid females ($n=61$) were found from late September to early February, and clutches ($n=26$) were recorded from November to February (Table 1). Hatchlings ($n=100$ individuals from 21 clutches) were born from early January to late April (Table 1), after incubation periods that lasted 58–90 days ($\bar{x}=80\pm 7$ days, $n=21$ clutches).

Clutch size and relative mass

The number of eggs varied from three to ten per clutch ($\bar{x}=5.3\pm 1.6$ eggs, $n=26$) and the number of vitellogenic follicles varied from three to 12 per female ($\bar{x}=5.3\pm 2.1$

follicles, $n=24$). There was no significant difference between the mean number of eggs per clutch and the mean number of follicles in secondary vitellogenesis per female ($t=0.30$, $df=48$, $P=0.976$). Female SVL explained 9% of the variation in the number of eggs or vitellogenic follicles ($r^2=0.09$, $F_{1,48}=4.50$, $P=0.039$); 24% of the variation in the mean mass of eggs per clutch was determined by maternal SVL ($r^2=0.24$, $F_{1,24}=7.42$, $P=0.012$). RCM ranged between 0.19 and 0.42 ($\bar{x}=0.34\pm 0.05$, $n=25$), and in 80% of observations ($n=20$) was above 0.30. Mean mass of eggs per clutch explained 67% of the variation in SVL, and 77% of the variation in body mass of hatchlings ($r^2=0.67$, $F_{1,19}=38.74$, $P<0.001$ and $r^2=0.77$, $F_{1,19}=64.37$, $P<0.001$, respectively). Maternal SVL explained 18% of the variation in SVL and body mass of hatchlings; the regressions were, however, only marginally significant ($r^2=0.18$, $F_{1,19}=4.23$, $P=0.054$ and $r^2=0.18$, $F_{1,19}=4.30$, $P=0.052$, respectively).

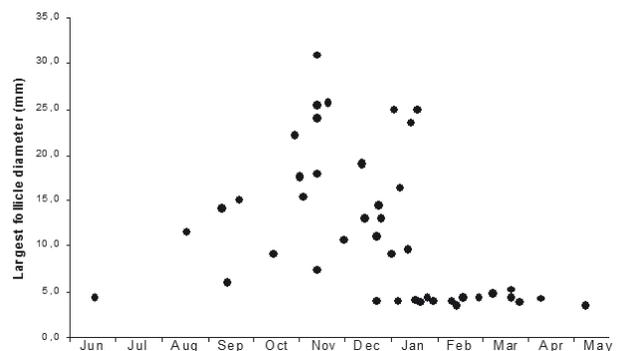


Fig. 4. Seasonal variation in the diameter of the largest ovarian follicles in mature female *Xenodon dorbignyi*.

Table 1. Seasonal occurrence of vitellogenesis, matings, gravid females, oviposition and hatching of *Xenodon dorbignyi*. "X" represents occurrence. Numbers represent observations, with numbers of clutches in parentheses where applicable.

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Vitellogenesis	X	X	X	X	X	X			
Matings	1	1	4	1	1				
Gravid females		1	15	31	11	2	1		
Oviposition				14	7	3	2		
Hatching						20 (4)	55 (11)	15 (4)	10 (2)

Reproductive frequency

The relative number of mature females found gravid in November in six consecutive years (1998–2003) varied from 46.7 to 80.0% (\bar{x} =65.5 %) (Table 2). Annual reproduction was observed in two females (field #136 and 1098) captured gravid in two consecutive reproductive seasons.

Female *X. dorbignyi* might produce more than one clutch per reproductive season. Female MCP 11405 was collected on 8 November 1999 and oviposited 14 days later. On 3 December, the specimen died, containing well developed, 10 mm long follicles. Two matings were recorded for females that had just laid eggs. On 2 November 2000, one female was found laying eggs buried in the sand, with four males close to her. All five individuals were collected and put together in a terrarium, where the female oviposited before mating with a male. Another female, captured gravid and kept in captivity until oviposition, was released on 25 November 2000, and was found mating the following morning. On two other occasions (6 and 15 November 1999), males were found courting gravid females who oviposited a few days later.

Table 2. Absolute and relative numbers of mature female *Xenodon dorbignyi* found gravid in November from 1998 to 2003.

Year	Number of gravid females	Total number of mature females	Percentage of gravid females
1998	5	7	71.4
1999	10	14	71.4
2000	9	12	75.0
2001	4	5	80.0
2002	7	15	46.7
2003	1	2	50.0
Total	36	55	65.5

One female was captured gravid (detected by palpation) on 1 November 2002, and recaptured, again gravid, on 1 January 2003.

DISCUSSION

Maturity and sexual dimorphism

Among Squamata, larger species tend to attain maturity at a smaller proportion of the maximum size (Shine & Charnov, 1992). For *X. dorbignyi*, the relative size of maturity was 0.52 and 0.38 for females and males, respectively. These values are low compared to *Liophis aesculapii* (♀ =0.74, ♂ =0.59; Marques, 1996), *L. poecilogyrus* (♀ =0.61, ♂ =0.57; Maciel, 2001), *X. merremii* (♀ =0.42, ♂ =0.57; Jordão, 1996) and *X. neuwiedii* (♀ =0.67, ♂ =0.47; Jordão, 1996), despite the fact that these species attain a larger SVL than *X. dorbignyi* (except for male *L. poecilogyrus*). As a general rule, male snakes attain sexual maturity before females, due to their smaller reproductive costs (Parker & Plummer, 1987).

Female snakes attain larger body size than conspecific males in the majority of species (Shine, 1993; for *Xenodontini* see Jordão, 1996; Marques, 1996; Maciel, 2001; Frota, 2005). Male and female *X. dorbignyi* are born with similar SVL, attain approximately the same body size at older ages and probably have similar growth patterns. Males usually attain equal or larger sizes than females in species with male combat (Shine, 1993). At present we have no evidence for combats in *X. dorbignyi*, and the lack of differences in body size reached by males and females may be due to an advantage for large males during reproductive aggregations.

That males have relatively larger tails than females is common in snakes (King, 1989; Shine, 1993). In species that form reproductive aggregations, male tail length is related to reproductive success, and linked to the ability to actively remove the tail of other males from the female's cloaca (Madsen & Shine, 1993; Shine et al., 1999). The large size of the tail in male *X. dorbignyi* may reflect the morphological differences related to the presence of the hemipenis described by King (1989). An advantage for males with longer tails in reproductive aggregations can also not be discarded.

In many snakes, females are heavier than males of the same length (Shine, 1993), which may be a result of fecundity selection. In *X. dorbignyi*, fecundity selection

may act towards the production of larger eggs, which result in larger hatchlings.

Reproductive cycle

The presence of well-developed follicles in a female collected in mid-August indicates that vitellogenesis begins in July, followed by rapid follicular development (Keogh et al., 2000). Seasonal reproduction is common in temperate snakes (Seigel & Ford, 1987). In southern Brazil, reproductive seasonality was recorded in all snakes, including the Xenodontini *Liophis flavifrenatus*, *L. jaegeri*, *L. miliaris*, *L. poecilogyrus*, *X. dorbignyi*, *X. merremii* and *X. neuwiedii* (e.g. Leitão-de-Araújo, 1978; Pontes & Di-Bernardo, 1988; Aguiar & Di-Bernardo, 2005; Balustrin & Di-Bernardo, 2005; Di-Bernardo et al., 2007). Aseasonal reproduction or extensive vitellogenesis was verified in several species of Xenodontini in northern Brazil (*Liophis aesculapii*: Marques, 1996; *L. poecilogyrus*: Vitt & Vangilder, 1983; Pinto & Fernandes, 2004; *L. reginae* and *L. typhlus*: Martins, 1994; *X. merremii*: Jordão, 1996; Vitt, 1983; *X. neuwiedii*: Jordão, 1996; Marques, 1998; *X. severus*: Dixon & Soini, 1986). According to Jordão (1996) and Marques (1996), species of Xenodontini are able to reproduce continually, although reproduction is constrained by environmental factors in some regions. Shine (1977) suggested that the synchronised reproductive cycles in temperate snakes are a result of temperature constraints on egg development, and also ensure that births occur when food availability is highest. The synchronization among the reproductive cycles of all oviparous snakes in Rio Grande do Sul suggests that environmental temperature is the factor that determines reproductive cycles in the region, although the diet of *X. dorbignyi* is locally composed of anurans that may be abundant only for a short period (Oliveira et al., 2001).

Clutch size and relative mass, and reproductive frequency

Our study suggests that all follicles generally developed into eggs. The correlation between the number of eggs and SVL was weak, whereas egg mass was strongly related to the female's SVL, possibly as a result of selective forces acting towards an increase in hatchling size rather than hatchling number. Oliveira et al. (2004) noted that predation on *X. dorbignyi* by *Athene cunicularia* (burrowing owl) was biased towards smaller individuals, confirming that larger hatchlings might have higher survival.

Vitt & Price (1982) demonstrated that RCM is lower in species that depend on mobility and velocity to capture their prey and/or to avoid predators. Seigel & Fitch (1984), however, revealed that only some species matched this prediction. *Xenodon dorbignyi* is a cryptic and active forager that moves slowly, feeds on inactive amphibians and shows elaborate defensive behaviour (Oliveira, 2001; Oliveira et al., 2001; Tozetti et al., 2009). RCM values are similar to, for example, members of the genus *Heterodon*, to which it is not very closely related (Zaher et al., 2009), despite morphological and ecological similarities. This suggests that RCM is probably determined by factors related to the species' ecology.

Only in two out of six years was the proportion of gravid females recorded in November equal to or lower than 50%. This is probably an underestimate, as oviposition starts in early November and the detection of pregnancy by palpation is only possible when the eggs are well developed; we therefore assume that most females reproduce annually. Multiple clutches in the same reproductive season have not yet been recorded for Brazilian snakes under natural conditions, despite the simultaneous occurrence of developed follicles and eggs in oviducts of Xenodontini (*Liophis aesculapii*: Marques, 1996; *L. viridis*: Vitt, 1983; *Xenodon merremii*: Di-Bernardo et al., 2007; *Xenodon neuwiedii*: Jordão, 1996), multiple clutches in captivity (*L. aesculapii*: Marques, 1996; *L. jaegeri*, *L. poecilogyrus* and *X. merremii*: Di-Bernardo, 1998), and individuals with follicles of three different size classes (*L. miliaris* and *X. neuwiedii*: Di-Bernardo, 1998). However, following Seigel & Ford (1987), the mere presence of vitellogenic follicles does not guarantee that they become eggs in the same reproductive season, and the occurrence of multiple clutches in captivity may be due to higher food availability. Di-Bernardo et al. (2007) suggested that multiple clutches might occur in natural conditions when food availability is high, and the follicular development rate is high enough to convert at least some of the well-developed follicles into eggs. The evidence of multiple clutches obtained from museum specimens and the records in captivity for Xenodontini species indicate the ability to produce multiple clutches. Mating soon after egg laying was recorded, as was a specimen with developed follicles just after having laid eggs. Several females that laid eggs in January and February may already have produced a clutch at the beginning of the reproductive season.

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APPENDIX 1

Specimens examined deposited in scientific collections

BRASIL: RIO GRANDE DO SUL: Arroio do Sal (MCP 2759, MCP 2760, MCP 5172), Balneário Pinhal (DZU-FRGS 1506, MCN 9158, MCP 2267, MCP 2351, MCP 2758, MCP 5174-5177, MCP 5180, MCP 5181, MCP 5563, MCP 10619, MCP 10947, MCP 12365, MCP 12478, MCP 12521, MCP 12522, MCP 12580, MCP 12668-12671, MCP 12714, MCP 12715, MCP 12738, MCP 12739, MCP 13190, MCP 13334, MCP 13902, MCP 14169-14173, MCP 14258, MCP 14259, MCP 14346, MCP 14428, MCP 14442, MCP 14443), Capão da Canoa (MCN 9356, MCP 6730, MCP 6731), Cidreira (MCN 2958, MCN 2977, MCN 3273, MCN 3274, MCN 3283, MCN 3329, MCN 4665, MCN 6561, MCN 6592, MCN 7304, MCP 1225, MCP 1667, MCP 5184, MCP 12020), Imbé (DZUFRGS 338, MCN 7132, MCN 8076, MCP 2681, MCP 5010), Osório (MCP 11405), Palmares do Sul (MCN 3689, MCN 3690, MCN 6201, MCP 10669, MCP 10670, MCP 10691, MCP 10737, MCP 10738, MCP 10846, MCP 10943-10946, MCP 10993, MCP 11050, MCP 11129, MCP 11130, MCP 11339, MCP 11429, MCP 12526), São José do Norte (MCP 10728, MCP 10729), Tavares (MCP 5064), Torres (MCN 6192, MCN 6194, MCN 6199, MCN 8099), Tramandaí (DZUFRGS 249, MCN 2750, MCN 6203, MCN 8118).

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